



RNA virus circulation among pollinator and non-pollinator species in Argentina

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ABSTRACT. A decline in pollinating insect populations has been detected in recent years. Its causal factors include pathogens, among which RNA viruses have been the most notable. Numerous insect species could act as reservoirs for these viruses, resulting in emerging infectious diseases for new hosts. To date, ten honey bee viruses have been identified in Argentina, some of which have been found in other pollinators and non-pollinators that share the environment. The objective of this study was to review the available scientific literature related to the presence and dispersion of RNA viruses in the Apoidea species in Argentina. After a first search, 178 citations were found using keywords such as 'RNA virus', 'insects', and 'Argentina', and redefining other keywords such as 'bees', 'pollinators' and 'non-pollinators', 30 citations were found. While this could lead to a decrease in the populations of the new hosts, the spread of RNA viruses could lead to emerging infectious diseases in them.

[Keywords: environment, honey bees, Picornavirales, transmission]

RESUMEN. Circulación de virus ARN entre diversas especies de polinizadores y no-polinizadores en la Argentina. En los últimos años se ha detectado un descenso en las poblaciones de insectos polinizadores. Los factores causales de estas disminuciones incluyen patógenos, entre los que se destacan los virus ARN. Numerosas especies de insectos podrían actuar como reservorio de estos virus, resultando en enfermedades infecciosas emergentes para nuevos hospedadores. A la fecha se han identificado 10 virus de abejas en la Argentina, algunos de los cuales fueron hallados en otros polinizadores y no-polinizadores que comparten el ambiente. El objetivo de este estudio fue recabar información científica relacionada con la presencia y dispersión de los virus ARN en especies de Apoideos en la Argentina. Luego de una primera búsqueda se encontraron 178 citas utilizando palabras claves como 'ARN virus', 'insectos' y 'Argentina', y al redefinir otras palabras claves como 'abejas', 'polinizadores' y 'no polinizadores' se encontraron 30 citas. Mientras que este escenario podría llevar a la disminución de las poblaciones del nuevo hospedador, la diseminación de virus ARN podría conducir a la aparición de enfermedades infecciosas emergentes en ellos.

[Palabras clave: ambiente, abejas melíferas, Picornavirales, transmisión]

INTRODUCTION

In recent decades, pollinator populations have undergone a drastic downward trend (Potts et al. 2010; Hallmann et al. 2017). There are a number of factors related to industrial modes of agriculture that affect colonies of bees and other pollinators around the world. These factors range from habitat degradation due to monocultures with a consequent decline in flowering plants and the use of harmful insecticides (Nicholls and Altieri 2013), to biological factors, including bacterial, parasitic and viral infections, which also play a major role, with negative impact on pollinator populations (Forfert et al. 2015; Fünfhaus et al. 2018; McMenamin and Flenniken 2018; Grozinger and Flenniken 2019).

Among pollinators, honey bees (*Apis mellifera*) play a key role for human society and in ecosystem maintenance and diversity. In addition to being prized for their honey and wax production (Gallai et al. 2009), honey bees are important to world agricultural production because several crops are pollinator-dependent (Aizen et al. 2009; vanEngelsdorp and Meixner 2010; Paudel et al. 2015), and a decrease in their abundance would compromise world agricultural production. The worldwide decline in honey bees has led to a phenomenon called Colony Collapse Disorder (CCD) or Hive Depopulation Syndrome (CDS). Its possible causes have been discussed by several authors around the world, including Argentina (Maggi et al. 2013; Requier et al. 2018), with current consensus of multifactorial origin, including

a set of pathogens among which viruses are outstanding (McMenamin and Genersch 2015; García et al. 2019). Recent research has shown the presence of some of these viruses in some insect species that cohabit the environment of pollinators. These insects constitute a potential viral reservoir, which could enable the viruses to spread and generate an emerging infectious disease (EID) in these new hosts. To shed light in this scenario, the aim of this study was to review the available scientific literature related to the presence and dispersion of RNA virus in the Apoidea species in Argentina. We searched for the keywords 'RNA virus', 'insects', 'pollinators', 'apiary' and 'hive' in the academic literature search engines Pubmed (NCBI), Scielo, Redalyc and Google Scholar.

In a first search, 178 citations using keywords such as 'RNA virus', 'insects' and 'Argentina' were found. Then, redefining the search with keywords such as 'bees and pollinators' and 'non-pollinators', 25 and 5 citations were found, respectively. The results enabled us to determine where honey bee viruses have been found inside and outside hives. Furthermore, we were able to determine the occurrence of honeybee viruses in hives infested by *Varroa* spp. and *Braula schmitzi*, and in other pollinators such as bumble bees, stingless bees and carpenter bees. We also included

in the review other arthropods found in the Apoidea species that do not fulfill a pollinating function, such as *Nezara viridula*.

VIRUSES IN ARGENTINIAN POLLINATOR AND NON-POLLINATOR SPECIES

Approximately, 70 bee viruses have been described to date worldwide, of which 65 are RNA and only 5 are DNA viruses (Remnant et al. 2017; Beaurepaire et al. 2020). The prevalence and pathogenicity of DNA viruses are almost unknown, in contrast to the knowledge of the RNA viruses that affect insects, particularly pollinators. Ten bee viruses have been detected to date in Argentina (Allen and Ball 1996; Sguazza et al. 2013; Brasesco et al. 2021a; Salina et al. 2021). Of these, *Apis mellifera filamentous virus* (AmFV) is the only DNA virus present in Argentina (Quintana et al. 2019), while the others are RNA viruses of the order Picornavirales, family Dicistroviridae (*Israeli Acute Paralysis Virus* [IAPV], *Acute Bee Paralysis Virus* [ABPV], *Black Queen Cell Virus* [BQCV]), family Iflavirus (Deformed Wing Virus [DWV A and DWV B], Sacbrood Bee Virus [SBV], and of the order Nodamuvirales, family Sinhaliviridae (*Lake Sinai Virus* [LSV]). The *Chronic Bee Paralysis Virus* (CBPV), the *Cloudy*

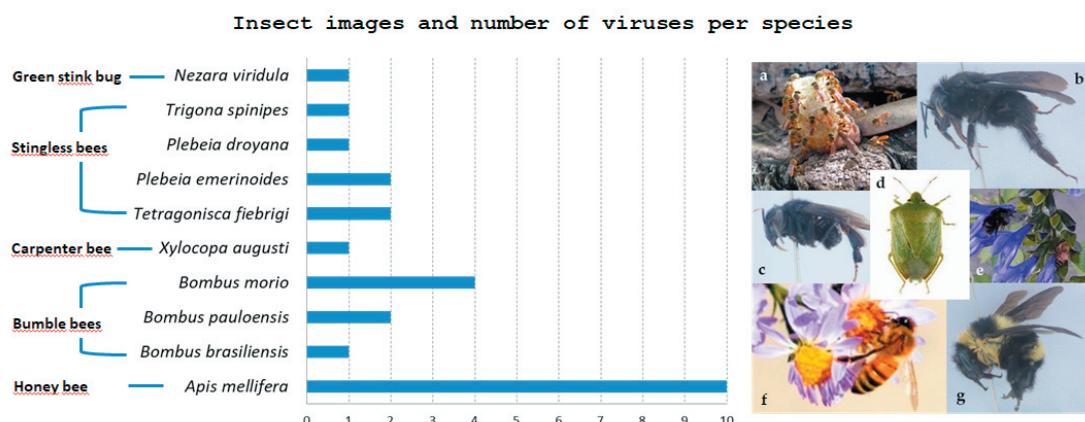


Figure 1. Number of viruses per species found in the insect groups analyzed from Argentina, according to Allen and Ball (1996), Reynaldi et al. (2013), Sguazza et al. (2013), Lucia et al. (2014), Álvarez et al. (2017), Molineri et al. (2017), Bravi et al. (2019), Quintana et al. (2019), Susevich et al. (2019), Brasesco et al. (2021 a, b), Salina et al. (2021), and images of insects detected positive for virus: (a) *Tetragonisca fiebrigi* in their nest, (b) worker of *Bombus pauloensis*, (c) *Trigona spinipes*, (d) *Nezara viridula*, (e) *Bombus pauloensis* and *Apis mellifera*, (f) worker of *Apis mellifera*, and (g) worker of *Bombus brasiliensis*.

Figura 1. Número de virus por especie encontrados en los grupos de insectos analizados de la Argentina, según Allen and Ball (1996), Reynaldi et al. (2013), Sguazza et al. (2013), Lucía et al. (2014), Álvarez et al. (2017), Molineri et al. (2017), Bravi et al. (2019), Quintana et al. (2019), Susevich et al. (2019), Brasesco et al. (2021 a,b), Salina et al. (2021), e imágenes de insectos detectados positivos para virus: (a) *Tetragonisca fiebrigi* en el nido, (b) obrera de *Bombus pauloensis*, (c) *Trigona spinipes*, (d) *Nezara viridula*, (e) *Bombus pauloensis* y *Apis mellifera*, (f) obrera de *Apis mellifera*, y (g) obrera de *Bombus brasiliensis*.

Table 1. RNA virus from bees found in insects from Argentina by molecular analysis. References: (+) positive samples, (-) negative samples, (nd) not detected, (IAPV) Israeli acute bee paralysis virus, (DWV) Deformed Wing Virus, (SBV) Sacbrood Bee Virus, (ABPV) Acute Bee Paralysis Virus, (BQCV) Black Queen Cell Virus, (CBPV) Chronic Bee Paralysis Virus, (BVX) Bee Virus X, (CWV) Cloudy Wing Virus, (LSV) Lake Sinai Virus, and (AmFV) *Apis mellifera* Filamentous Virus.

Tabla 1. Virus ARN de abejas hallados en insectos de Argentina mediante análisis moleculares. Referencias: (+) muestras positivas, (-) muestras negativas, (nd) no detectado, (IAPV) Virus Israelí de la Parálisis Aguda, (DWV) Virus de las Alas Deformadas, (SBV) Virus de la Cría Ensacada, (ABPV) Virus de la Parálisis Aguda, (BQCV) Virus de las Celdas Reales Negras, (CBPV) Virus de la Parálisis Crónica, (BVX) Virus X de las Abejas, (CWV) Virus de las Alas Nubosas, (LSV) Virus del Lago Sinaí, y (AmFV) Virus Filamentoso de *Apis mellifera*.

| | | IAPV | DWV | SBV | ABPV | BQCV | CBPV | BVX | CWV | LSV | AmFV | References |
|---------------|---|------|-----|-----|------|------|------|-----|-----|-----|------|---|
| Honey bee | <i>Apis mellifera</i> (L.) 1758 | + | + | + | + | + | + | + | + | + | + | Allen and Ball (1996), Sguazza et al. (2013), Molineri et al. (2017), Quintana et al. (2019), Brasesco et al. (2021 a, b), Salina et al. (2021) |
| Bumble bees | <i>Bombus brasiliensis</i> (Lepeletier 1836) | + | - | - | - | - | - | - | nd | nd | nd | nd |
| | <i>Bombus pauloensis</i> (ex <i>atratus</i>) (Friese 1913) | + | - | - | + | - | - | - | nd | nd | nd | Bravi et al. (2019), Reynaldi et al. (2013) |
| | <i>Bombus morio</i> (Swederus 1787) | + | + | + | + | - | - | - | nd | nd | nd | Bravi et al. (2019) |
| Carpenter bee | <i>Xylocopa augusti</i> (Lepeletier 1841) | - | + | - | - | - | - | - | nd | nd | nd | Lucía et al. (2014) |

Wing Virus (CWV) and the Bee Virus X (BVX) have not yet been classified (ICTV 2021).

Worldwide, the most prevalent viruses are DWV and SBV (Remnant et al. 2017), while in Argentina, the most prevalent are DWV, IAPV and SBV (Molineri et al. 2017). These viruses have been recorded in honey bees and in many other groups of pollinators in Argentina (Figure 1). Reynaldi et al. (2013) reported the first infection of *Bombus pauloensis* (Friese 1913) (ex *atratus* Franklin 1913) (Hymenoptera: Apidae), and Bravi et al. (2019) found these viruses in *Bombus brasiliensis* (Lepeletier 1836), *B. pauloensis* (Friese 1913) and *Bombus morio* (Swederus 1787). Stingless bees (Álvarez et al. 2017) and the carpenter bee *Xylocopa augusti* (Lepeletier 1841; Lucía et al. 2014) were also found positive for these viruses. Furthermore, honey bee viruses have been described in ants (Formicidae) (Sébastien et al. 2015) and IAPV has also been identified in a non-hymenopteran insect, the green stink bug *Nezara viridula* L. 1758 (Susevich et al. 2019). Even though Manley et al. (2015) proposed

that the RNA virus spillover is more likely to occur when the hosts are phylogenetically close, recent studies report that they have been detected in several insect orders, including Araneae, Blattodea, Coleoptera, Dermaptera, Odonata and Orthoptera. Dobelmann et al. (2020) and Levitt et al. (2013) suggest that even if infected insects are phylogenetically distant from *A. mellifera*, they could act as viral reservoirs, resulting in the dispersion of pathogens towards honey bees and the rest of the pollinators, as proposed by Reynaldi et al. (2013) for bumble bees (Table 1).

NON-POLLINATOR INSECTS AS HUBS OF RNA VIRUS DISPERSION

Varroa destructor mite is considered the main parasite of *A. mellifera* worldwide (Rosenkranz et al. 2010). For this reason, honey bees use different strategies to natural control this infestation. Between them, grooming behavior, that involves the physical removal of Varroa mites from adult honey bees, is one of the most

studied. This behavior could be autogrooming, where parasitized bees can dislodge mites themselves using their legs and mandibles or allogrooming where honey bees receive help from other honey bees to take them out (Russo et al. 2020). This situation made Varroa one of the cohabitants of the hive.

More than forty years ago, honey bee viruses were found in the ectoparasitic mites *Varroa destructor* (Ball 1983). To date, there are several reports of honey bee viruses being transmitted by Varroa mites, including DWV (Bowen-Walker et al. 1999), KBV (Chen et al. 2004; Shen et al. 2005a), SBV (Shen et al. 2005b), ABPV (Ball 1983) and IAPV (Di Prisco et al. 2011). The spread of Varroa mites around the world thus facilitated the dispersal of several viruses. Particularly, in infection with DWV, the mite can act as a vector for the viral pathogen and trigger its replication in infected bees (Villalobos 2016). Parallel to its role as a viral vector, it has been shown to influence the immune status of its host. The globalization of *V. destructor* and the concomitant DWV infections have raised the question of what influence DWV has on the health of bee colonies. The association between DWV and *Varroa destructor* has been identified as one of the main causes of loss of bee colonies worldwide (Annoscia et al. 2019). On the other hand, several authors have reported that ABPV or BQCV increase their prevalence in the absence of Varroa mites (Lin et al. 2018).

Recently, in Ecuador, the bee louse *Braula schmitzi* was found positive for ABPV (Avalos et al. 2019). The detection of ABPV in colonies parasitized by *B. schmitzi* could indicate the possibility of a new host within the hive, changing the view of this insect in the colony. This dipteran was believed to have been eliminated in Argentina since 1990. However, a focal outbreak in three apiaries of the Magdalena district, Buenos Aires province, was reported in 2015 by the Bee Health Laboratory of the Ministry of Agroindustry of Buenos Aires Province (Pérez Raúl, personal communication). Therefore, it is important to be alert to the presence of different insects that could infest honey bees and act as hubs of RNA viruses.

VIRUS RESERVOIRS AND NEW HOSTS

Several authors suggested the importance of flowers in the transmission of honey bee pathogens, including bee viruses (Singh et

al. 2010; Dalmon et al. 2021). According to that, viral transmission among species can be mediated by different routes, like shared flowers (Alger et al. 2019) while infected bees visit them and possibly contaminate their surface, nectar and pollen with viral particles, leading to an indirect virus transmission (Singh et al. 2010; Bodden et al. 2019)

The characteristics of the RNA single-stranded viruses suggest that they have a high mutation rate, resulting in virus populations with high variation amongst genotypes (Holmes 2009). Moreover, the geographic proximity between pollinators and the high contact with food sources would provide a high level of adaptability, enabling viruses to change hosts in nature and cause disease outbreaks that could devastate wild pollinator populations (Woolhouse et al. 2005; Li et al. 2011).

The presence of different viral infections in both honey bees and organisms that share resources with them is important because these organisms can act as natural virus reservoirs in addition to being new host populations. Knowledge of virus prevalence in the environment and of transmission mechanisms would enable the development of contingency plans for controlling colony losses and population decline according to each situation. Moreover, although honey bee colonies seldom die as a direct result of a viral infection, they are often weakened by it (Neumenn and Carreck 2010; Watson and Stallins 2016; Steinhauer et al. 2018). Similar effects have been observed on bumble bee colonies, causing debilitation and even death (Cameron and Sadd 2020).

FUTURE PERSPECTIVES

Ecological differences among host species can be related to the prevalence of infections. Nest dynamics, as well as biological and behavioral aspects, differ among bee species, as they do with other organisms. Therefore, it is necessary to analyze the mechanisms and direction of the transmission of these viruses among different arthropod species in order to understand their dynamics within arthropod communities and to determine the role of arthropods as potential mechanical vectors, reservoirs or hosts.

DECLARATION OF CONFLICT OF INTERESTS .
The authors have no conflict of interest to declare.

ACKNOWLEDGMENTS. We would like to thank Leopoldo J. Álvarez and Gerardo Martí for their contribution to this paper and to the anonymous referees. The study was financed

by the projects V260 National University of La Plata (UNLP) and PICT 2017 -1046 National Agency for Scientific and Technological Promotion (FONCyT).

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